

**This document represents the current approved pharmacology –toxicology section of the NCI/CIP IND for  $^{89}\text{Zr}$ -Panitumumab.**

**This IND was permitted by FDA to proceed in an early trial without requiring specific toxicology studies on the modified antibody. The approach used in this section may be used for other small dose  $^{89}\text{Zr}$ -antibodies, provided that there is sufficient data to establish that there is minimal risk to patients as determined by the FDA. “Sufficient” data, for example, could be the full pharmacology/toxicology package required for the therapeutic antibody, to which you have a letter of reference, as well as confirming *in vitro* and *in vivo* data on the biological activity of the modified antibody.**

## 8. PHARMACOLOGY AND TOXICOLOGY DATA

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## 8.1 Introduction

The imaging drug is panitumumab, labeled with deferoxamine (DFO) and <sup>89</sup>Zr. Panitumumab and deferoxamine are approved drugs with substantial human exposure. The usual dose of panitumumab is 6 mg/kg every two weeks, 420 mg/70 kg patient; the usual dose of deferoxamine is 50 mg/kg/day, approximately 3.5-5 gm. Both drugs are administered by slow intravenous infusion, panitumumab over 1 hour for doses less than 1000 mg and deferoxamine at no more than 15 mg/kg/hour, 1050 mg/hr/70 kg patient. Thus for these drugs, the administration rate is 7-17 mg in the first minute of infusion. The total dose of <sup>89</sup>Zr-panitumumab will be less than one 1 mg (less than 7 nmole), and is therefore about 1/420 of the panitumumab therapeutic dose and 1/1000 of the deferoxamine first hour dose. At this micro-dose, no toxicity is expected to be observed, but patients will be closely monitored for any adverse effects.

The immunoreactivity and specificity of the radiolabeled panitumumab was confirmed in vitro, the biodistribution was determined in normal mice and the radiation doses were calculated. Imaging of mice with implanted xenograft tumors having low, medium and high expression of EGFR established that the labeled drug accumulated in the tumors according to the measure expression level in the tumors.

The known toxicity profile of the two components, deferoxamine and panitumumab, the previous experience in humans with antibodies labeled with <sup>89</sup>Zr by the same methodology and the pre-clinical studies all support the conclusion that a trial of labeled panitumumab at a microdose of 1 mg, approximately 1/420 of the usual clinical dose, will not pose a significant risk to the patients in the proposed early clinical development.

## 8.2 Pharmacology and Toxicology of Panitumumab and deferoxamine

The imaging drug is panitumumab, labeled with deferoxamine (DFO) and <sup>89</sup>Zr. Panitumumab and deferoxamine are approved drugs with substantial human exposure. The usual dose of panitumumab is 6 mg/kg, 420 mg/70 kg patient; the usual dose of deferoxamine is 50 mg/kg/day, approximately 3.5-5 gm. Both drugs are administered by slow intravenous infusion, panitumumab over 1 hour for doses less than 1000 mg and deferoxamine at no more than 15 mg/kg/hour, 1050 mg/70 kg patient. Thus for these drugs, the administration rate is 7-17 mg in the first minute of infusion. The total dose of <sup>89</sup>Zr-panitumumab will be less than one 1 mg (less than 7 nmole), and is therefore about 1/420 of the panitumumab therapeutic dose and 1/1000 of the deferoxamine first hour dose. At this micro-dose, no toxicity is expected to be observed. However, for the sake of completeness, the text below details the safety information for each drug at its normal therapeutic dose.

1. **Panitumumab:** Panitumumab (Vectibix®) is an FDA-approved drug, an epidermal growth factor receptor antagonist indicated as a single agent for the treatment of metastatic colorectal carcinoma with disease progression on or following fluoropyrimidine, oxaliplatin, and irinotecan chemotherapy regimens. It is approved in Canada as monotherapy for the treatment of patients

with EGFR expressing metastatic colorectal carcinoma (mCRC) with non-mutated (wild-type) *KRAS* after failure of fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy regimens. In the European Union, the approval includes indications for the treatment of patients with wild-type *KRAS* metastatic colorectal cancer (mCRC) in first-line in combination with FOLFOX and in second-line in combination with FOLFIRI in patients who have received first-line fluoropyrimidine-based chemotherapy (excluding irinotecan).

a. FDA Summary Basis of Approval for PLS#125147 (panitumumab)

The pharmacology & toxicological section of the FDA summary basis of approval for BLA#125147<sup>1</sup> is . The verbatim summary of that section is:

*"A. Brief overview of nonclinical findings.* Panitumumab (ABX-EGF, AMG 954; VECTIBIX™) was evaluated for pharmacologic activity in human tumor cell lines *in vitro* and in human tumor xenografts in nude mice, and for toxicity and pharmacokinetics in nude mice and cynomolgus monkeys. Tissue binding studies demonstrated that ABX-EGF bound VEGFR with moderate to strong intensity to surface epidermal growth factor receptor (EGFR) in samples of both human and cynomolgus monkey skin, tonsil, breast, and prostate, and in urothelium of the ureter and urinary bladder, and uterine endometrium and cervical squamous epithelium in monkeys. Treatment of tumor-bearing nude mice with panitumumab alone or in combination with several different biologic or chemotherapy regimens resulted in delayed tumor growth in human colon, epidermoid, breast, or pancreatic cancers. Where effective, combination therapy with panitumumab and selected chemotherapy or biologic anti-tumor treatments resulted in approximately additive, but not synergistic effects. Pharmacokinetic profiles of panitumumab in cynomolgus monkeys following initial, i/v injections of 7.5, 15, 30, or 60 mg/kg doses showed linear, dose-related increases in  $C_{max}$  and  $AUC_{0-t}$ , dose-related decreases in clearance with a concomitant increase in apparent elimination half-life, and steady state volumes of distribution approximately equal to the plasma space. Steady state, as evidenced by peak and trough serum ABX-EGF levels was achieved in repeat dose studies following approximately 5 to 6 doses of panitumumab. With repeated administration for 4 to 26 weeks, the dose-related decreases in clearance and increases elimination half-life were slightly higher than following the initial dose; however, the  $C_{max}$  and  $AUC_{0-last}$  were only slightly (< 2-fold) increased over the initial, observed values. Therefore, the toxicokinetic evaluations confirmed that exposure to ABX-EGF was continuous over the duration of these studies with little accumulation of drug. Although group mean values for  $C_{max}$  and  $AUC_{0-last}$  were frequently not different for the same dose levels of ABX-EGF over the study durations, anti-panitumumab antibodies developed in several monkeys in all repeat-dose studies, resulting in decreased ABX-EGF exposure in these individual animals, and in some cases, reversal of some of the panitumumab-related toxicities. Severe dermatologic and gastrointestinal toxicities were noted at all dose levels in cynomolgus monkeys treated weekly with 7.5, 15, 30, or 60 mg/kg panitumumab for 4, 13, or 26 weeks. These doses correspond to approximately 1.25 to 10-fold greater than the proposed human dose of 6 mg/kg ABX-EGF administered every two weeks, and approximately 3 to 24-fold higher than the proposed 2.5

mg/kg/week panitumumab dose, when adjusted for body weight. Observed toxicities included decreases in body weight and food consumption, decreases in serum calcium, phosphate, and magnesium, and dose-dependent clinical signs consisting of soft or watery stool, alopecia, skin rash, erythema, flaking and/or dryness, suppurative dermatitis, erosions, sloughing, and ulcerations, and in several studies, early mortalities secondary to the severity of the skin lesions. These changes occurred with increased frequency and severity as both the dose and duration of ABX-EGF increased, and only partially reversible following discontinuation of panitumumab treatment. Panitumumab treatment inhibited ovarian function in non-pregnant female monkeys, and was abortifacient, although not teratogenic when administered to pregnant animals from GD20 through GD48, throughout organogenesis.

*B. Pharmacologic activity.* VECTIBIX™ binding to the EGFr competitively inhibits the binding of its normal ligands including EGF and transforming growth factor- $\alpha$ , which are implicated in tumor growth, and stimulates receptor internalization, leading to a reduction of EGFr expression on the cell surface. This antagonist action inhibits phosphorylation and activation of EGFr-associated kinases, resulting in inhibition of cell growth, and decreased vascular endothelial growth factor, interleukin-8, and other growth factor production. The epidermal growth factor receptor (EGFr) is constitutively expressed in many normal epithelial tissues, including the skin follicle, placenta, and mammary gland. Over-expression of EGFr is also detected in many human cancers including those of the colon and rectum. *In vitro* assays and *in vivo* animal studies have shown that VECTIDIX™, alone or in combination with irinotecan, but not fluorouracil, oxaliplatin, or cisplatin chemotherapy inhibits the growth and survival of several human tumor cells that overexpress the EGFr. No anti-tumor effects of panitumumab were observed in immune deficient mouse models bearing human tumor xenografts with levels of EGFr expression below 10,000 receptors per cell, suggesting that a threshold level of EGFr expression is required for tumor response to VECTIDIX™ to occur.

*C. Nonclinical safety issues relevant to clinical use.* The dermatologic toxicities following panitumumab treatment were observed both in cynomolgus monkeys following repeat administration, and in clinical trials of ABX-EGF in patients with metastatic, colorectal cancer. In the non-human primate models, dermatologic toxicities included severe erythema, skin flaking, scaling and sloughing, pustule formation, infections, and erosions or ulcerations. Early mortalities were observed in several studies secondary to the severe skin lesions, and the incidence and timing were related to the dose of ABX-EGF. These doses correspond to approximately 1.25 to 10-fold greater than the proposed human dose of 6 mg/kg ABX-EGF administered every two weeks, and approximately 3 to 24-fold higher than the 2.5 mg/kg/week panitumumab dose, when adjusted for body weight. The dermatologic toxicities observed in these studies are consistent with the pharmacodynamic effects of ABXEGF in inhibiting critical intracellular pathways involved with the activation and function of EGFr expressed on skin cells, and subsequent inhibition of epidermal cell growth and maturation. Skin lesions, including-acneform rash, pruritus, dry skin, exfoliation, skin fissures, and paronychia were also observed clinically in approximately 90% of 789 metastatic colorectal cancer patients.

treated with panitumumab. Dermatologic toxicities in these patients were generally Grade 2 in severity, with approximately 12% of subjects (95/789) reporting Grade 3 or higher skin changes. Although not observed in the nonclinical toxicity studies, Grade 1/2 stomatitis and oral mucositis were also reported in approximately 7% of these patients. Development of severe dermatologic toxicity occasionally resulted in infectious complications including sepsis and in rare occasions, death, but most frequently led to either panitumumab dose interruption or dose modification. The clinical skin toxicities have been adequately described in the WARNINGS and ADVERSE REACTIONS sections of the proposed packaged insert; however, additional modifications to these sections to include the findings in cynomolgus monkeys will be requested by the reviewer. Panitumumab treatment of non-pregnant, female cynomolgus monkeys inhibited ovarian function, resulting in dose-related irregularities in menstrual cycling (prolonged menstrual cycles and/or amenorrhea), decreased pregnancy rates, and decreases in serum 17 $\beta$ -estradiol and progesterone levels, at doses corresponding to approximately 1.25 to 5-fold higher than the proposed, clinical doses when adjusted for body weight. Although no teratogenic effects were observed, panitumumab was abortifacient at all dose levels tested in pregnant female cynomolgus monkeys, following weekly injection from GD20 through GD48 (approximately 1.25 to 6-fold greater than the highest proposed human dose). While these findings may not be relevant to the indicated, clinical population (metastatic colorectal cancer), they have been included in the PRECAUTIONS section of the panitumumab label. However, additional language regarding both the fertility and developmental effects of panitumumab treatment is included in Appendix 1 of this review, for communication to the sponsor.

Hypomagnesemia, hypocalcemia, and hypophosphatemia were observed in several of the nonclinical, repeat-dose toxicity studies of ABX-EGF in cynomolgus monkeys, and have also been reported in clinical trials of panitumumab. These toxicities may be secondary to the moderate to severe diarrhea and dehydration observed in both the non-human primate studies, and in metastatic colorectal patients treated with panitumumab, alone or in combination with irinotecan and 5-fluorouracil chemotherapy. The electrolyte disturbances and diarrhea have been adequately described in the section of the proposed package insert for VECTIBIX™, and recommendations for periodic monitoring of patients for hypomagnesemia and hypocalcemia during and for 8 weeks following completion of panitumumab are included in the label under Laboratory Monitoring, in the PRECAUTIONS section.

Clinical toxicities not predicted by the animal studies included infusion reactions in < 2% of panitumumab treated, colorectal cancer patients, occurring within 24 hours of the first dose. No patients had life-threatening or fatal, ABX-EGF associated infusion reactions, and the events were reported as severe in only 5/789 (<1 %) patients. Most of the potential infusion reactions were mild in intensity, resolved without treatment, were isolated occurrences and did not require alteration or interruption of panitumumab dosing. The infusion reactions have been identified in the section of the package insert, and require no further action from the pharmacology and toxicology review staff. “

b. The Vectibix package insert<sup>2</sup>

The US package insert is attached as an appendix. Vectibix labeling has a “black box” warning for dermatologic toxicity and infusion reactions. In the Warnings and Precautions section, the verbatim text:

**1 Dermatologic Toxicity**

In Study 1, dermatologic toxicities occurred in 90% of patients and were severe (NCI-CTC grade 3 and higher) in 16% of patients with mCRC receiving Vectibix. The clinical manifestations included, but were not limited to, dermatitis acneiform, pruritus, erythema, rash, skin exfoliation, paronychia, dry skin, and skin fissures.

Subsequent to the development of severe dermatologic toxicities, infectious complications, including sepsis, septic death, necrotizing fasciitis, and abscesses requiring incisions and drainage were reported. Withhold Vectibix for severe or life-threatening dermatologic toxicity [*see Boxed Warning, Adverse Reactions (6.1), and Dosage and Administration (2.1)*].

**2 Infusion Reactions**

In Study 1, 4% of patients experienced infusion reactions and in 1% of patients, these reactions were graded as severe (NCI-CTC grade 3-4).

Infusion reactions, manifesting as anaphylactoid reactions, bronchospasm, and hypotension, can occur following Vectibix administration [*see Boxed Warning and Adverse Reactions (6.1, 6.3)*]. In clinical studies, severe infusion reactions occurred with the administration of Vectibix in approximately 1% of patients. Fatal infusion reactions occurred in postmarketing experience. Terminate the infusion for severe infusion reactions [*see Dosage and Administration (2.1)*].

**3 Increased Toxicity With Combination Chemotherapy**

Vectibix is not indicated for use in combination with chemotherapy. In an interim analysis of Study 2, the addition of Vectibix to the combination of bevacizumab and chemotherapy resulted in decreased overall survival and increased incidence of NCI-CTC grade 3-5 (87% vs 72%) adverse reactions [*see Clinical Studies (14)*]. NCI-CTC grade 3-4 adverse drug reactions occurring at a higher rate in Vectibix-treated patients included rash/dermatitis acneiform (26% vs 1%), diarrhea (23% vs 12%), dehydration (16% vs 5%), primarily occurring in patients with diarrhea, hypokalemia (10% vs 4%), stomatitis/mucositis (4% vs < 1%), and hypomagnesemia (4% vs 0). NCI-CTC grade 3-5 pulmonary embolism occurred at a higher rate in Vectibix-treated patients (7% vs 4%) and included fatal events in three (< 1%) Vectibix-treated patients.

As a result of the toxicities experienced, patients randomized to Vectibix, bevacizumab, and chemotherapy received a lower mean relative dose intensity of each chemotherapeutic agent (oxaliplatin, irinotecan, bolus 5-FU, and/or infusional 5-FU) over the first 24 weeks on study, compared with those randomized to bevacizumab and chemotherapy.

In a single-arm study of 19 patients receiving Vectibix in combination with IFL, the incidence of NCI-CTC grade 3-4 diarrhea was 58%; in addition, grade 5 diarrhea occurred in one patient. In a single-arm study of 24 patients receiving Vectibix plus FOLFIRI, the incidence of NCI-CTC grade 3 diarrhea was 25%.

Severe diarrhea and dehydration, which may lead to acute renal failure and other complications, have been observed in patients treated with Vectibix in combination with chemotherapy.

#### 4 Pulmonary Fibrosis/Interstitial Lung Disease (ILD)

Pulmonary fibrosis occurred in less than 1% (2/1467) of patients enrolled in clinical studies of Vectibix. Patients with a history or evidence of interstitial pneumonitis, pulmonary fibrosis, were excluded from most clinical trials. Vectibix® (panitumumab)

Therefore, the estimated risk in a general population that includes such patients is uncertain. Cases of interstitial lung disease (ILD), including fatalities, have been reported in patients treated with Vectibix. Interrupt Vectibix therapy for the acute onset or worsening of pulmonary symptoms. Discontinue Vectibix therapy if ILD is confirmed.

#### 5 Electrolyte Depletion/Monitoring

In Study 1, median magnesium levels decreased by 0.1 mmol/L in the Vectibix arm; hypomagnesemia (NCI-CTC grade 3 or 4) requiring oral or intravenous electrolyte repletion occurred in 2% of patients. Hypomagnesemia occurred 6 weeks or longer after the initiation of Vectibix. In some patients, both hypomagnesemia and hypocalcemia occurred. Patients' electrolytes should be periodically monitored during and for 8 weeks after the completion of Vectibix therapy. Institute appropriate treatment, e.g., oral or intravenous electrolyte repletion, as needed.

- 2. Deferoxamine:** Deferoxamine (Desferal®) is a drug approved throughout much of the world for the treatment of acute iron intoxication and of chronic iron overload due to transfusion-dependent anemias. Deferoxamine chelates iron by forming a stable complex that prevents the iron from entering into further chemical reactions. It readily chelates iron from ferritin and hemosiderin but not readily from transferrin; it does not combine with the iron from cytochromes and hemoglobin. Deferoxamine does not cause any demonstrable increase in the excretion of electrolytes or trace metals. In this use as an imaging drug, the chelate site is occupied by the <sup>89</sup>Zr and is not displaced by serum iron.



a. Approval documents

The first approval in the US was in 1968 and there is no summary basis of approval.

b. The Desferal Package Insert<sup>3</sup>

Desferal does not have a “black box” warning. The section on Adverse Reactions is repeated here verbatim: The following adverse reactions have been observed, but there are not enough data to support an estimate of their frequency.

**At the Injection Site:** Localized irritation, pain, burning, swelling, induration, infiltration, pruritus, erythema, wheal formation, eschar, crust, vesicles, local edema. Injection site reactions may be associated with systemic allergic reactions (see Body as a Whole, below).

**Hypersensitivity Reactions and Systemic Allergic Reactions:** Generalized rash, urticaria, anaphylactic reaction with or without shock, angioedema.

**Body as a Whole:** Local injection site reactions may be accompanied by systemic reactions like arthralgia, fever, headache, myalgia, nausea, vomiting, abdominal pain, or asthma. Infections with *Yersinia* and *Mucormycosis* have been reported in association with Desferal use (see PRECAUTIONS).

**Cardiovascular:** Tachycardia, hypotension, shock.

**Digestive:** Abdominal discomfort, diarrhea, nausea, vomiting.

**Hematologic:** Blood dyscrasia (thrombocytopenia, leucopenia).

**Hepatic:** Increased transaminases, hepatic dysfunction.

**Musculoskeletal:** Muscle spasms. Growth retardation and bone changes (e.g., metaphyseal dysplasia) are common in chelated patients given doses above 60 mg/kg, especially those who begin iron chelation in the first three years of life. If doses are kept to 40 mg/kg or below, the risk may be reduced .

**Nervous System:** Neurological disturbances including dizziness, peripheral sensory, motor, or mixed neuropathy, paresthesias, seizures; exacerbation or precipitation of aluminum-related dialysis encephalopathy.

**Special Senses:** High-frequency sensorineural hearing loss and/or tinnitus are uncommon if dosage guidelines are not exceeded and if dose is reduced when ferritin levels decline. Visual disturbances are rare if dosage guidelines are not exceeded. These may include decreased acuity, blurred vision, loss of vision, dyschromatopsia, night

blindness, visual field defects, scotoma, retinopathy (pigmentary degeneration), optic neuritis, and cataracts.

**Respiratory:** Acute respiratory distress syndrome (with dyspnea, cyanosis, and/or interstitial infiltrates) (see WARNINGS). **Skin:** Very rare generalized rash. **Urogenital:** Dysuria, acute renal failure, increased serum creatinine and renal tubular disorders.

**Postmarketing Reports** There are postmarketing reports of deferoxamine-associated renal dysfunction, including renal failure. Monitor patients for changes in renal function (e.g., increased serum creatinine).

### 8.3. Previous human exposure to <sup>89</sup>Zr-antibodies.

Full peer-reviewed publications are noted for two different <sup>89</sup>Zr-antibodies, trastuzumab (HER2 targeted) and U36 (CD44v6 targeted) and are discussed below. However, 15 studies are currently listed on [clinicaltrials.gov](https://clinicaltrials.gov)<sup>4</sup>, 6 with labeled bevacizumab, 3 with labeled trastuzumab, 2 with labeled cetuximab, and 4 with different monoclonal antibodies. To the best of our knowledge, all of these studies use the same DFO labeling strategy. The list is shown below. (Additional publications are now available for citation, please review the literature and add newer clinical studies here)

- 1 Recruiting [Clinical and Molecular Correlates of Positron Emission Tomography \(PET\) With <sup>89</sup>Zr-DFO-huJ591 in Metastatic Prostate Cancer](#)  
Condition: Prostate Cancer  
Intervention: Drug: <sup>89</sup>Zr-DFO-huJ591
- 2 Not yet recruiting [<sup>89</sup>Zr-RO5323441 PET Imaging in Glioblastoma](#)  
Condition: Glioblastoma  
Intervention: Radiation: Molecular imaging with <sup>89</sup>Zr-RO5323441
- 3 Recruiting [Imaging HSP90 Inhibitor AUY922 on VEGF-<sup>89</sup>Zr-bevacizumab Positron Emission Tomography \(PET\)](#)  
Condition: Breast Cancer  
Intervention: Other: <sup>89</sup>Zr-bevacizumab PET imaging
- 4 Completed [<sup>89</sup>Zr-trastuzumab PET for Imaging the Effect of HSP90 Inhibition](#)  
Condition: Breast Cancer  
Intervention: Other: Imaging with <sup>89</sup>Zr-trastuzumab PET
- 5 Completed [<sup>89</sup>Zr-bevacizumab PET Imaging in Patients With Neuroendocrine Tumors](#)  
Condition: Neuroendocrine Tumors  
Interventions: Drug: <sup>89</sup>Zr-bevacizumab; Drug: Everolimus
- 6 Recruiting [Safety and Imaging Study of GC1008 in Glioma](#)  
Condition: Primary Brain Tumors  
Interventions: Other: <sup>89</sup>Zr-GC1008; Drug: GC1008

- |    |                    |   |
|----|--------------------|---|
| 7  | Recruiting         | <a href="#"><u>Vascular Endothelial Growth Factor (VEGF) Imaging Before and During Everolimus Treatment for Renal Cell Carcinoma</u></a><br>Conditions: Metastatic Renal Cell Carcinoma; Everolimus Treatment<br>Intervention: Other: 89Zr-bevacizumab PET scan   |
| 8  | Completed          | <a href="#"><u>VEGF Imaging in Renal Cell Carcinoma</u></a><br>Condition: Renal Cell Carcinoma<br>Intervention: Other: 89Zr-Bevacizumab PET-scan  |
| 9  | Completed          | <a href="#"><u>VEGF Early Imaging for Breast Cancer</u></a><br>Condition: Breast Cancer<br>Intervention: Other: 89Zr-bevacizumab PET  |
| 10 | Recruiting         | <a href="#"><u>Visualizing Vascular Endothelial Growth Factor (VEGF) Producing Lesions in Von Hippel-Lindau Disease</u></a><br>Conditions: Von Hippel-Lindau Disease; Hemangioblastoma; Renal Cell Carcinoma; Pheochromocytoma; Pancreatic Neuroendocrine Tumor<br>Intervention: Other: 89Zr bevacizumab PET scan |
| 11 | Recruiting         | <a href="#"><u>A Study of RO5429083 in Patients With Metastatic and/or Locally Advanced, CD44-Expressing, Malignant Solid Tumors</u></a><br>Condition: Neoplasms<br>Intervention: Drug: RO5429083   |
| 12 | Recruiting         | <a href="#"><u>HER2 Imaging Study to Identify HER2 Positive Metastatic Breast Cancer Patient Unlikely to Benefit From T-DM1</u></a><br>Condition: HER-2 Positive Breast Cancer<br>Interventions: Drug: T-DM1; Procedure: 89Zr-trastuzumab   |
| 13 | Recruiting         | <a href="#"><u>Pilot Imaging Study With 89Zr-Trastuzumab in HER2-positive Metastatic Breast Cancer Patients</u></a><br>Conditions: Breast Neoplasms; Secondary; HER2 Positive Carcinoma of Breast<br>Intervention: Drug: Zr89-trastuzumab   |
| 14 | Recruiting         | <a href="#"><u>Non-invasive Imaging of Cetuximab-Zr. 89 Uptake Wit PET: a Phase I Trial in Stage IV Cancer Patients</u></a><br>Condition: Stage I V Cancer<br>Intervention: Drug: Cetuximab-Zr.   |
| 15 | Not yet recruiting | <a href="#"><u>Adaptive Radiation Treatment for Head and Neck Cancer</u></a><br><b>Condition:</b> Head and Neck Cancer<br><b>Interventions:</b> Drug: cisplatinum; Radiation: radiotherapy; Drug: Cetuximab   |

The published abstracts of peer-reviewed papers using Zr-labeled antibodies in human studies are as follows (all but the Rizvi paper use the same chelating moiety):

**1. Radiation dosimetry of <sup>89</sup>Zr-labeled chimeric monoclonal antibody U36 as used for immuno-PET in head and neck cancer patients.<sup>5</sup>**

**PURPOSE:** Immuno-PET is an appealing concept in the detection of tumors and planning of antibody-based therapy. For this purpose, the long-lived positron emitter (<sup>89</sup>Zr (half-life, 78.4 h) recently became available. The aim of the present first-in-humans (<sup>89</sup>Zr immuno-PET study was to assess safety, biodistribution, radiation dose, and quantification of the (<sup>89</sup>Zr-labeled chimeric monoclonal antibody (cmAb) U36 in patients with head and neck squamous cell carcinoma (HNSCC). In addition, the performance of immuno-PET for detecting lymph node metastases was evaluated, as described previously.

**METHODS:** Twenty HNSCC patients, scheduled to undergo surgical tumor resection, received 75 MBq of (<sup>89</sup>Zr-cmAb U36 (10 mg). Immuno-PET scans were acquired at 1, 24, 72, or 144 h after injection. The biodistribution of the radioimmunoconjugate was evaluated by ex vivo radioactivity measurement in blood and in biopsies from the surgical specimen obtained at 168 h after injection. Uptake levels and residence times in blood, tumors, and organs of interest were derived from quantitative immuno-PET studies, and absorbed doses were calculated using OLINDA/EXM 1.0. The red marrow dose was calculated using the residence time for blood.

**RESULTS:** (<sup>89</sup>Zr-cmAb U36 was well tolerated by all subjects. PET quantification of blood-pool activity in the left ventricle of the heart showed a good agreement with sampled blood activity (difference equals 0.2% +/- 16.9% [mean +/- SD]) except for heavy-weight patients (>100 kg). A good agreement was also found for the assessment of mAb uptake in primary tumors (mean deviation, -8.4% +/- 34.5%). The mean absorbed red marrow dose was 0.07 +/- 0.02 mSv/MBq and 0.09 +/- 0.01 mSv/MBq in men and women, respectively. The normal organ with the highest absorbed dose was the liver (mean dose, 1.25 +/- 0.27 mSv/MBq in men and 1.35 +/- 0.21 mSv/MBq in women), thereafter followed by kidneys, thyroid, lungs, and spleen. The mean effective dose was 0.53 +/- 0.03 mSv/MBq in men and 0.66 +/- 0.03 mSv/MBq in women. Measured excretion via the urinary tract was less than 3% during the first 72 h.

**CONCLUSION:** (<sup>89</sup>Zr immuno-PET can be safely used to quantitatively assess biodistribution, uptake, organ residence times, and radiation dose, justifying its further clinical exploitation in the detection of tumors and planning of mAb-based therapy.

## **2. Performance of immuno-positron emission tomography with zirconium-89-labeled chimeric monoclonal antibody U36 in the detection of lymph node metastases in head and neck cancer patients.<sup>6</sup>**

**PURPOSE:** Immuno-positron emission tomography (PET), the combination of PET with monoclonal antibodies (mAb), is an attractive option to improve tumor detection and to guide mAb-based therapy. The long-lived positron emitter zirconium-89 ((<sup>89</sup>Zr) has ideal physical characteristics for immuno-PET with intact mAbs but has never been used in a clinical setting. In the present feasibility study, we aimed to evaluate the diagnostic imaging performance of immuno-PET with (<sup>89</sup>Zr)-labeled-chimeric mAb (cmAb) U36 in patients with squamous cell carcinoma of the head and neck (HNSCC), who were at high risk of having neck lymph node metastases.

**EXPERIMENTAL DESIGN:** Twenty HNSCC patients, scheduled to undergo neck dissection with or without resection of the primary tumor, received 75 MBq (<sup>89</sup>Zr) coupled to the anti-CD44v6 cmAb U36 (10 mg). All patients were examined by computed tomography (CT) and/or magnetic resonance imaging (MRI) and immuno-PET before surgery. Six patients also underwent PET with (18)F-fluoro-2-deoxy-d-glucose. Immuno-PET scans were acquired up to 144 hours after injection. Diagnostic findings were recorded per neck side (left or right) as well as per lymph node level (six levels per side), and compared with histopathologic outcome. For this purpose, the CT/MRI scores were combined and the best of both scores was used for analysis.

**RESULTS:** Immuno-PET detected all primary tumors (n = 17) as well as lymph node metastases in 18 of 25 positive levels (sensitivity 72%) and in 11 of 15 positive sides (sensitivity 73%). Interpretation of immuno-PET was correct in 112 of 121 operated levels (accuracy 93%) and in 19 of 25 operated sides (accuracy 76%). For CT/MRI, sensitivities of 60% and 73% and accuracies of 90% and 80% were found per level and side, respectively. In the six patients with seven tumor-involved neck levels and sides, immuno-PET and (18)F-fluoro-2-deoxy-d-glucose PET gave comparable diagnostic results.

**CONCLUSION:** In this study, immuno-PET with (<sup>89</sup>Zr)-cmAb U36 performed at least as good as CT/MRI for detection of HNSCC lymph node metastases.

## **3. Biodistribution of <sup>89</sup>Zr-trastuzumab and PET imaging of HER2-positive lesions in patients with metastatic breast cancer.<sup>7</sup>**

We performed a feasibility study to determine the optimal dosage and time of administration of the monoclonal antibody zirconium-89 ((<sup>89</sup>Zr)-trastuzumab to enable positron emission tomography (PET) imaging of human epidermal growth factor receptor 2 (HER2)-positive lesions. Fourteen patients with HER2-positive metastatic breast cancer received 37 MBq of (<sup>89</sup>Zr)-trastuzumab at one of three doses (10 or 50 mg for those who were trastuzumab-naïve and 10 mg for those who were already on trastuzumab treatment). The patients underwent at least two PET scans between days 2 and 5. The results of the study showed that the best time for assessment of (<sup>89</sup>Zr)-trastuzumab uptake by tumors was 4-5 days after the injection. For optimal PET-scan results, trastuzumab-naïve patients required a 50 mg dose of (<sup>89</sup>Zr)-trastuzumab, and patients already on trastuzumab treatment required a 10 mg dose. The accumulation of (<sup>89</sup>Zr)-trastuzumab in lesions allowed PET imaging of most of the known lesions

and some that had been undetected earlier. The relative uptake values (RUVs) (mean  $\pm$  SEM) were 12.8  $\pm$  5.8, 4.1  $\pm$  1.6, and 3.5  $\pm$  4.2 in liver, bone, and brain lesions, respectively, and 5.9  $\pm$  2.4, 2.8  $\pm$  0.7, 4.0  $\pm$  0.7, and 0.20  $\pm$  0.1 in normal liver, spleen, kidneys, and brain tissue, respectively. PET scanning after administration of (<sup>89</sup>Zr)-trastuzumab at appropriate doses allows visualization and quantification of uptake in HER2-positive lesions in patients with metastatic breast cancer.

#### **4. Zirconium-89-trastuzumab positron emission tomography as a tool to solve a clinical dilemma in a patient with breast cancer.<sup>8</sup>**

This is a case report of a patient with a difficult to access metastasis from one of two primary tumors, one HER2 positive and the other HER2 negative. Determining optimal treatment required knowing which primary had metastasized.

The following paper is a Zr-89 labeled antibody, but it uses a different chelating moiety than DFO.

#### **5. Biodistribution, radiation dosimetry and scouting of <sup>90</sup>Y-ibritumomab tiuxetan therapy in patients with relapsed B-cell non-Hodgkin's lymphoma using <sup>89</sup>Zr-ibritumomab tiuxetan and PET.<sup>9</sup>**

**PURPOSE:** Positron emission tomography (PET) with (<sup>89</sup>Zr)-ibritumomab tiuxetan can be used to monitor biodistribution of (<sup>90</sup>Y)-ibritumomab tiuxetan as shown in mice. The aim of this study was to assess biodistribution and radiation dosimetry of (<sup>90</sup>Y)-ibritumomab tiuxetan in humans on the basis of (<sup>89</sup>Zr)-ibritumomab tiuxetan imaging, to evaluate whether co-injection of a therapeutic amount of (<sup>90</sup>Y)-ibritumomab tiuxetan influences biodistribution of (<sup>89</sup>Zr)-ibritumomab tiuxetan and whether pre-therapy scout scans with (<sup>89</sup>Zr)-ibritumomab tiuxetan can be used to predict biodistribution of (<sup>90</sup>Y)-ibritumomab tiuxetan and the dose-limiting organ during therapy.

**METHODS:** Seven patients with relapsed B-cell non-Hodgkin's lymphoma scheduled for autologous stem cell transplantation underwent PET scans at 1, 72 and 144 h after injection of ~70 MBq (<sup>89</sup>Zr)-ibritumomab tiuxetan and again 2 weeks later after co-injection of 15 MBq/kg or 30 MBq/kg (<sup>90</sup>Y)-ibritumomab tiuxetan. Volumes of interest were drawn over liver, kidneys, lungs, spleen and tumours. Ibritumomab tiuxetan organ absorbed doses were calculated using OLINDA. Red marrow dosimetry was based on blood samples. Absorbed doses to tumours were calculated using exponential fits to the measured data.

**RESULTS:** The highest (<sup>90</sup>Y) absorbed dose was observed in liver ( $3.2 \pm 1.8$  mGy/MBq) and spleen ( $2.9 \pm 0.7$  mGy/MBq) followed by kidneys and lungs. The red marrow dose was  $0.52 \pm 0.04$  mGy/MBq, and the effective dose was  $0.87 \pm 0.14$  mSv/MBq. Tumour absorbed doses ranged from 8.6 to 28.6 mGy/MBq. Correlation between predicted pre-therapy and therapy organ absorbed doses as based on (<sup>89</sup>Zr)-ibritumomab tiuxetan images was high (Pearson correlation coefficient  $r = 0.97$ ). No significant difference between pre-therapy and therapy tumour absorbed doses was found, but correlation was lower ( $r = 0.75$ ).

**CONCLUSION:** Biodistribution of (89)Zr-ibritumomab tiuxetan is not influenced by simultaneous therapy with (90)Y-ibritumomab tiuxetan, and (89)Zr-ibritumomab tiuxetan scout scans can thus be used to predict biodistribution and dose-limiting organ during therapy. Absorbed doses to spleen were lower than those previously estimated using (111)In-ibritumomab tiuxetan. The dose-limiting organ in patients undergoing stem cell transplantation is the liver.

## 8.4 Preclinical studies with <sup>89</sup>Zr-Panitumumab

### 8.3.1 Immunoreactivity and specificity of <sup>89</sup>Zr-panitumumab

The immunoreactivity of the <sup>89</sup>Zr-panitumumab was assessed in a radioimmunoassay, as detailed in the literature<sup>10</sup>, using methanol-fixed cells. In brief, serial dilution of <sup>89</sup>Zr-protein (~200,000–30,000 cpm in 50 µL of BSA/PBS) were added to small test tubes containing MDA-MB-468 cells (1X10<sup>6</sup>/50 µL of BSA/PBS) from ‘NCI 60 cell screen’. Following 2 hr incubation at 37 °C, the cells were washed, pelleted, and counted in a γ-scintillation counter. The percentage of binding was calculated for each dilution and averaged. It was observed that 65% ±5% of the radioactivity was bound.

The specificity of the radiolabeled panitumumab was confirmed by incubation of one set of cells with radiolabeled panitumumab with 10 µg unlabeled panitumumab. Assay results showed that only 3.5±0.5% of radioactivity was bound, demonstrating that binding was specific.

Batch Number MBR-89Zr-PAN	Immunoreactivity (%)	Specificity (Absorbed radioactivity after blocking)(%)
04-17-12-001	65% ±5	3.5 ±0.5
04-18-12-001	68%±4	4.0±0.8
04-25-12-001	66%±4	3.0±0.5

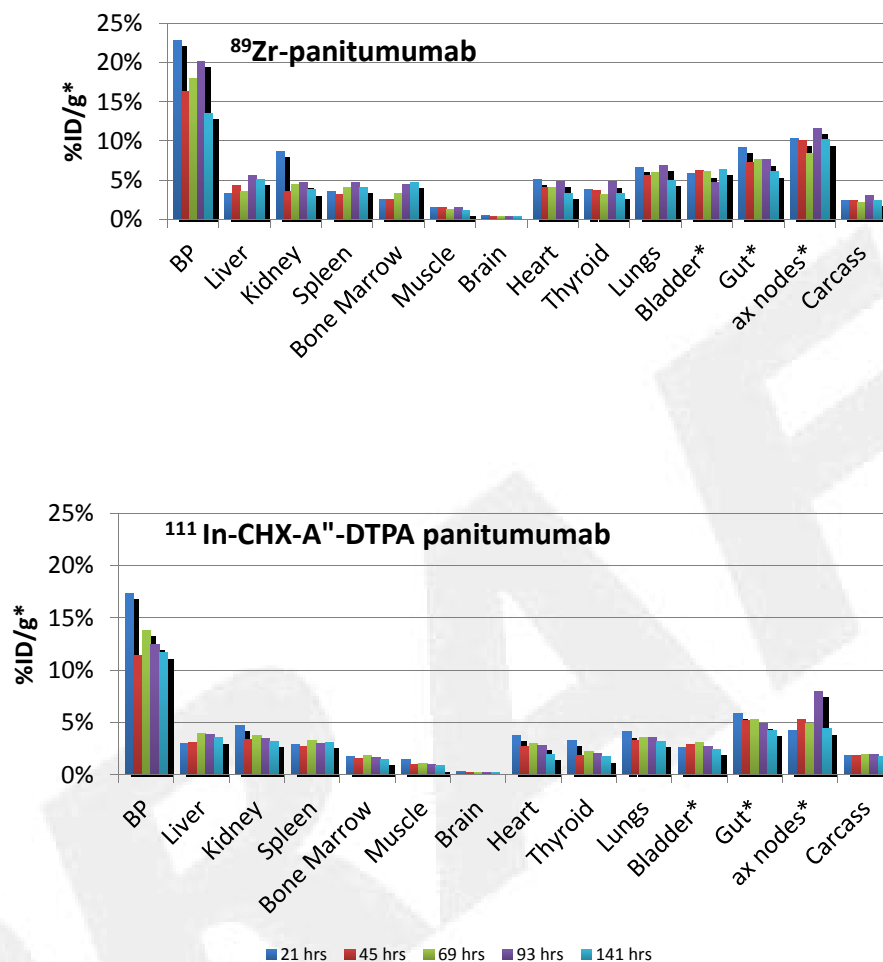
### 8.3.2. In vivo studies.

#### 8.3.2.1. Biodistribution and dosimetry in non-tumor bearing mice

Biodistribution studies were performed on non-tumor bearing athymic nude mice (Charles River Laboratories Inc, Frederick, MD). 1.85 MBq of <sup>89</sup>Zr-panitumumab formulated in 200 µL of 0.9% saline was administered (i.v. tail-vein injection) to each mouse. Mice were then sacrificed at different time-points (21, 45, 69, 93, and 141 hr) to measure the uptake in different organs: spleen, stomach, sm. intestine, ceacum, colon, bladder, liver, kidney, testis or ovary, uterus, axillary. lymph, heart, lung, salivary gland, thyroid, brain, skin, muscle, femur, tail, carcass. For select organs, %ID/g integrated over time and mouse to man organ scaling factors were used to calculate the total number of disintegrations (residence time). OLINDA/EXM 1.1 was then used to estimate human dosimetry. The biodistribution was compared to a different labeled panitumumab, <sup>111</sup>In-CHX-A''-panitumumab, used in pre-clinical SPECT imaging.

**Results:** Biodistribution is shown in Figure 1 as % injected dose within the organ. Note similar distribution of <sup>111</sup>In and <sup>89</sup>Zr panitumumab and persistent high blood counts (panitumumab t<sub>1/2</sub> =

7.5 days). High and increasing uptake within the axillary lymph nodes is consistent with the lymphatic system being a primary route of elimination for panitumumab.



**Figure 1. Biodistribution of <sup>89</sup>Zr and <sup>111</sup>In labeled panitumumab**

The calculated dosimetry is shown below in Table 1. The absorbed dose is relatively high, which will limit the total dose that can be administered to humans to 1.0-1.5 mCi. The human studies reported in the literature have used 1 or 2 mCi and achieved good image quality.



**Table 1. Dosimetry for <sup>89</sup>Zr-Panitumumab**

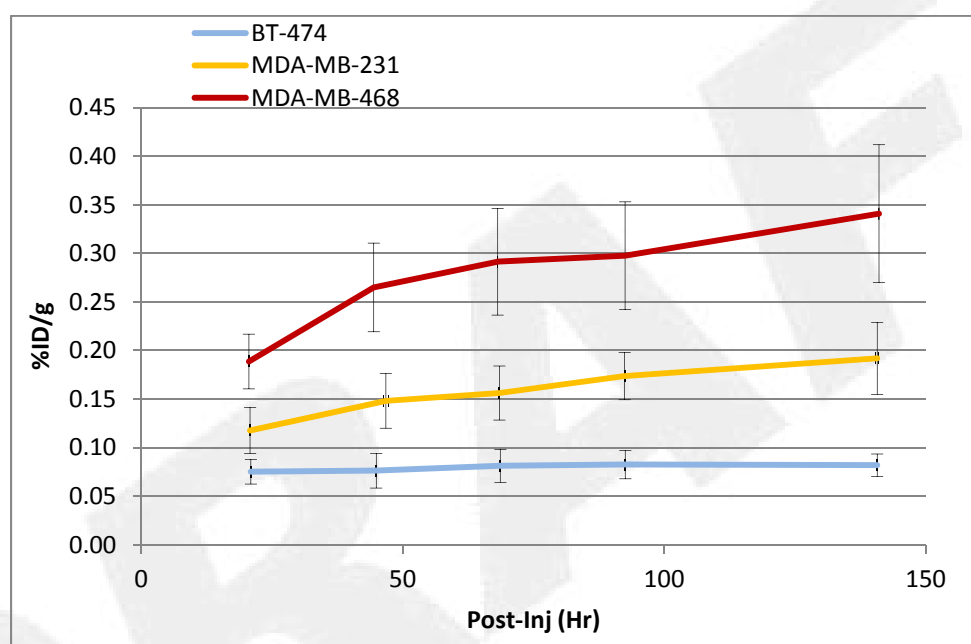
Target Organ	mSv/MBq	rad/mCi
Adrenals	0.599	2.220
Brain	0.212	0.785
Breasts	0.391	1.450
Gallbladder	0.522	1.930
LLI	0.711	2.630
Small	0.480	1.770
Stomach	0.460	1.700
ULI	0.549	2.030
Heart	2.900	10.700
Kidneys	0.541	2.000
Liver	0.723	2.680
<b>Lungs</b>	<b>0.953</b>	<b>3.530</b>
Muscle	0.402	1.490
Ovaries	0.476	1.760
Pancreas	0.578	2.140
<b>Red Marrow</b>	<b>0.816</b>	<b>3.020</b>
Osteogenic	0.677	2.500
Skin	0.243	0.901
Spleen	0.725	2.680
Testes	0.293	1.080
Thymus	0.737	2.730
Thyroid	0.353	1.310
Urinary Bladder	0.418	1.550
Uterus	0.435	1.610
Total	0.434	1.600
Effective Dose Equivalent	0.769	2.840
<b>Effective Dose</b>	<b>0.578</b>	<b>2.140</b>

### 8.3.2.2. Imaging Studies in tumor bearing mice.

For imaging studies BT-474, MDA-MB-231, and MDA-MB-468 xenografts were generated in athymic nude mice following subcutaneous injection of  $1 \times 10^7$  cells and corresponded to very low (Her1 negative), mid and high Her1 protein expression levels. Mice were administered <sup>89</sup>Zr-panitumumab ( $10.18 \pm 1.24$  MBq, 60-70 µg of mAb in 200 µL of 0.9% saline) via tail-vein injection when tumors reached 5 mm diameter. Animals remained conscious and were allowed free access to food and water prior to and after radiopharmaceutical injection. Animal imaging experiments were conducted on an Inveon micro-PET/CT scanner (Siemens Medical Solutions USA, Inc., Knoxville, TN) at 24,

48, 72, 96, and 144 hours post injection. During PET/CT scanning, mice were anesthetized (2% isoflurane in O<sub>2</sub> at 1 L/min) and imaged in the prone position for 5 minutes (CT) followed by 30 minutes (PET). Images were analyzed using ASIPro software; version 6.8.0.0 (Siemens Medical Solutions USA, Knoxville, TN).

**Results:** The uptake in the tumors with time is shown in Figure 2, representative images in Figure 3 and the determination of comparative EGFR expression by Western blot on the cell lines used for the xenografts is shown in Figure 4.



**Figure 2. Tumor Time-Activity Analysis, decay corrected**

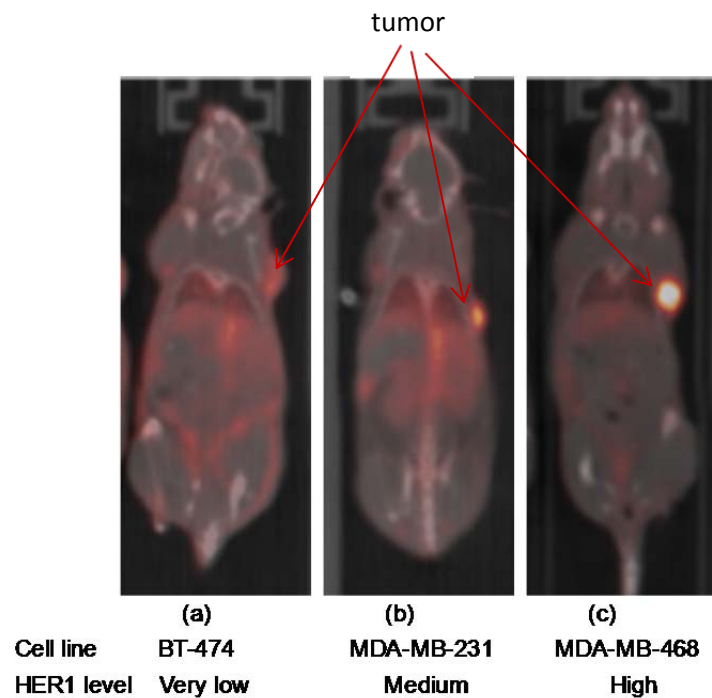


Figure 3. Representative images at 96 hours post injection

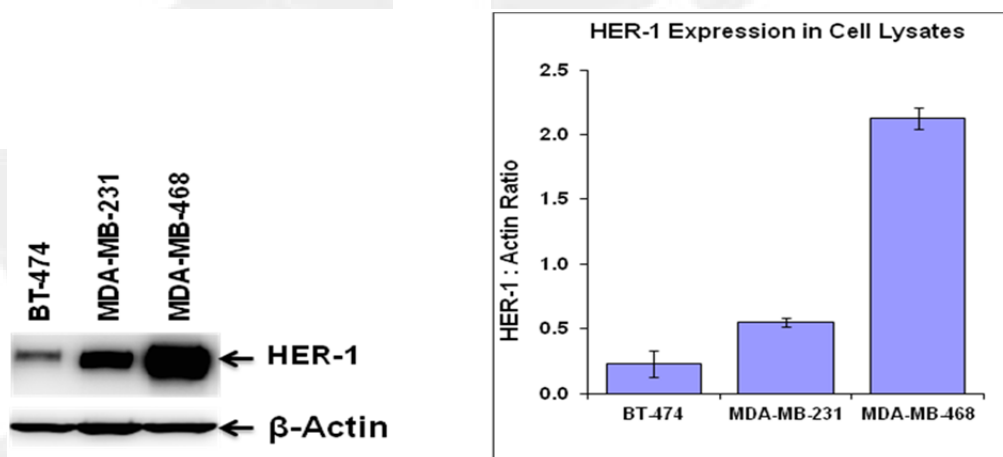


Figure 4. Determination of comparative EGFR expression level of BT-474, MDA-MB-231, and MDA-MB-468 cell lines by western blot analysis

### 8.3.2. Summary of preclinical studies

The immunoreactivity and specificity of the radiolabeled panitumumab was confirmed in vitro, the biodistribution was determined in normal mice and the radiation doses were calculated. Imaging of mice with implanted xenograft tumors having low, medium and high expression of EGFR established that the labeled drug accumulated in the tumors according to the measure expression level in the tumors.

## 8.5 Conclusion

The known toxicity profile of the two components, deferoxamine and panitumumab, the previous experience in humans with antibodies labeled with <sup>89</sup>Zr by the same methodology and the pre-clinical studies all support the conclusion that a trial of labeled panitumumab at a mass dose of 1 mg, less than 7 nmole, approximately 1/420 of the usual clinical dose, will not pose a significant risk to the patients in the proposed early clinical development.

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<sup>1</sup> Available via this link: [http://www.accessdata.fda.gov/drugsatfda\\_docs/nda/2006/125147s0000\\_PharmR.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/nda/2006/125147s0000_PharmR.pdf)  
Accessed 8/1/2012

<sup>2</sup> Accessible via this link: [http://www.accessdata.fda.gov/drugsatfda\\_docs/label/2009/125147s080lbl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/125147s080lbl.pdf)  
(accessed 8/1/2012)

<sup>3</sup> Accessible via this link: [http://www.accessdata.fda.gov/drugsatfda\\_docs/label/2011/016267s050lbl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/016267s050lbl.pdf).  
Accessed 8/1/2012

<sup>4</sup> Accessible via this link: <http://clinicaltrials.gov/ct2/results?term=89Zr> Accessed on 8/1/2012

<sup>5</sup> Börjesson PK, Jauw YW, de Bree R, Roos JC, Castelijns JA, Leemans CR, van Dongen GA, Boellaard R. [Radiation dosimetry of 89Zr-labeled chimeric monoclonal antibody U36 as used for immuno-PET in head and neck cancer patients.](#) J Nucl Med. 2009 Nov;50(11):1828-36.

<sup>6</sup> Börjesson PK, Jauw YW, Boellaard R, de Bree R, Comans EF, Roos JC, Castelijns JA, Vosjan MJ, Kummer JA, Leemans CR, Lammertsma AA, van Dongen GA. [Performance of immuno-positron emission tomography with zirconium-89-labeled chimeric monoclonal antibody U36 in the detection of lymph node metastases in head and neck cancer patients.](#) Clin Cancer Res. 2006 Apr 1;12(7 Pt 1):2133-40.

<sup>7</sup> Dijkers EC, Oude Munnink TH, Kosterink JG, Brouwers AH, Jager PL, de Jong JR, van Dongen GA, Schroder CP, Lub-de Hooge MN, de Vries EG. [Biodistribution of 89Zr-trastuzumab and PET imaging of HER2-positive lesions in patients with metastatic breast cancer.](#) Clin Pharmacol Ther. 2010 May;87(5):586-92. Epub 2010 Mar 31.

<sup>8</sup> Gaykema SB, Brouwers AH, Hovenga S, Lub-de Hooge MN, de Vries EG, Schröder CP. [Zirconium-89-trastuzumab positron emission tomography as a tool to solve a clinical dilemma in a patient with breast cancer.](#) J Clin Oncol. 2012 Feb 20;30(6):e74-5. Epub 2011 Dec 27.

<sup>9</sup> Rizvi SN, Visser OJ, Vosjan MJ, van Lingen A, Hoekstra OS, Zijlstra JM, Huijgens PC, van Dongen GA, Lubberink M. [Biodistribution, radiation dosimetry and scouting of 90Y-ibritumomab tiuxetan therapy in patients with relapsed B-cell non-Hodgkin's lymphoma using 89Zr-ibritumomab tiuxetan and PET](#) Eur J Nucl Med Mol Imaging. 2012 Mar;39(3):512-20. Epub 2012 Jan 5.

<sup>10</sup> Nayak TK, Garmestani K, Milenic DE, Brechbiel MW. (2012) PET and MRI of Metastatic Peritoneal and Pulmonary Colorectal Cancer in Mice with Human Epidermal Growth Factor Receptor 1-Targeted <sup>89</sup>Zr-Labeled Panitumumab. *J Nucl Med.* 53(1):113-20.

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